DOCKET NO: 289779US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
MASATOSHI TOHATA, ET AL.	: EXAMINER: POPA, I.
SERIAL NO: 10/578,613	:
FILED: MARCH 12, 2007	: GROUP ART UNIT: 1633
FOR: RECOMBINANT MICROORGANISM	:

REPLY BRIEF

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

This Reply Brief responds to the Examiner's Answer ("EA") mailed March 18, 2011. The Appellants reiterate their arguments set forth in the Appeal Brief and respond to major issues raised in the EA. It is the Examiner's burden to show both a suggestion in the prior art for the invention as well as a reasonable expectation of success, *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). For the invention, that would constitute a showing that the prior art would have both motivated one to delete the *rocR* or *sigL* genes in an organism that expresses a heterologous protein to enhance the expression of the heterologous protein and that enhanced heterologous protein expression was nothing more than a predictable outcome.

The prior art does not expressly disclose or suggest deleting *rocR* or *sigL* from a recombinant microorganism that expresses a heterologous protein. However, the Examiner contends Ferrari suggests deleting genes under the control of *rocR* or *sigL* for the purpose enhancing heterologous protein expression. None of the genes listed by Ferrari are *rocR* or

sigL. Nevertheless, the Examiner believes that the *rocA*, *rocD* and *rocF* genes[1] are named by Ferrari as genes that might be deleted or inactivated to enhance heterologous protein expression and contends that these genes could be inactivated by deleting *rocR* or *sigL* which according to the secondary references Gardan, et al. (see abstract) encode the transcription activator RocR. Some prominent defects in the Examiner's arguments are as follows:

(i) No suggestion. None of the prior art references expressly suggests deleting or inactivating *rocR* or *sigL* in a recombinant cell that expresses a heterologous protein.

(ii) No reasonable expectation of success. Assuming that one of ordinary skill in the art would have sought to inactivate *rocA*, *rocD* or *rocF* by deleting or inactivating *rocR* or *sigL* (instead of directly deleting these genes) none of the prior art provided a reasonable expectation of success for enhancing heterologous protein expression by doing so.

(a) No objective data. While Ferrari mentions *rocA*, *rocD* or *rocF* as potential targets[2], it does not provide any examples or experimental data showing that inactivating these genes would have any effect on heterologous protein expression[3]. Thus, even were Ferrari read in light of Gardan to have suggested the invention, these references still would not have provided a reasonable expectation of success for enhancing heterologous protein expression by indirectly inactivating *rocA*, *rocD* or *rocF* by deleting or inactivating *sigL* or *rocR*.

---

[1] Inactivation of *rocADF* could inhibit the conversion of arginine into other amino acids and thus enhance consumption of arginine *per se*, but in doing so the consumption of amino acids made from arginine would be correspondingly decreased. The prior art does not suggest or explain why deletion of these genes would increase heterologous protein expression.

[2] Amongst an number of other potential target genes: *Sbo, slr, ybcO, csn, spoIISA, sigB, phrC, rapA, CssS, trpA, trpB, trpC, trpD, trpE, trpF, tdh/kbl, alsD, sigD, prpC, gapB, pckA, fbp,* ***rocA****, ycgN, ycgM,* ***rocF****, and* ***rocD****.*

[3] Example 1 describes *Creation of Deletion Strains* but is silent about how to create a strain having *rocA*, *rocD* and/or *rocF* deleted. Table 2 on pages 78-79 of Ferrari describes unique restriction enzyme pairs to make deletion constructs for Sbo, Slr, YbcO, and Csn, but does not mention *rocA*, *rocD* and/or *rocF*. Similarly, Table 4 on pages 81-82 does not refer to *rocA*, *rocD* and/or *rocF*. Examples 3-5 also are silent about *rocA*, *rocD* and/or *rocF* deletions and Figs. 7-8, which depict increased heterologous protein expression in some mutant strains, does not describe any mutants with deletions of *rocA*, *rocD* or *rocF*. Moreover, Figs. 7-8 show that certain deletions either had negative or minimal effects on levels of heterologous protein expression, e.g., see results for *sbo* and *slr* mutants in Table 7.

(b) Unpredictability of outcome due to known genetic complexity. One of ordinary skill in the art also would not have had a reasonable expectation of success of predictably enhancing heterologous protein synthesis in a recombinant cell by deleting *sigL* or *rocR* because these deletions would have been expected to have unpredictable, broad, global effects on cellular metabolism. That is, effects that go beyond those of just deleting *rocA*, *rocD* and/or *rocF*. Contrary to some of the Examiner's contentions (EA, page 7, lines 16-17), these broad global effects were recognized by the very prior art cited in the rejection. Gardan, page 825, col. 2, lines 16-18 teach "The positive regulatory protein RocR is required for the expression of both operons [*rocABC* and *rocDEF*]". When read as a whole Gardan suggests that deleting or inactivating *rocR* would have inhibited expression of ***all*** the proteins encoded by the *rocABC* and *rocDEF* operons[4]. A prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984). Thus, Gardan as a whole teaches away from a reasonable expectation of success for increasing heterologous protein expression since the effects of deleting ***all*** the genes in these two operons (or other genes under the control of *rocR* or *sigL*) would have been unpredictable.

Furthermore, Ferrari when considered as a whole teaches away from deleting *rocR* or *sigL* because these genes were well-known at the time the Ferrari application was filed[5], but were excluded from the Markush group of target genes theorized as being useful for increasing heterologous protein expression: *Expressio unius est exclusio alterius* ("the

[4] As explained in the Appeal Brief, global inactivation of *rocABCDEF* would have been expected to decrease arginine import and lead to a decrease in arginine accumulation in a microorganism because expression of *rocC* and *rocE* are relevant to arginine import as described by Gargan, et al. Thus, one would have had no reasonable expectation of success for increasing intracellular arginine concentration and heterologous protein expression since inactivation of *rocABCDEF* could result in the opposite effect to that of rocADF deletion and even if one of ordinary skill in the art knew that inactivation of *rocR* or *sigL* impaired *rocABCDEF* he or she would not have expected that the deletion of *rocR* or *sigL* would have enhanced heterologous protein synthesis.
[5] Gardan which describes SigL and RocR was published in 1997; Ferrari was filed in 2003.

express mention of one thing excludes all others"). One of ordinary skill in the art would have reasonably inferred from the exclusion of *sigL* and *rocR* from the Ferrari target gene list that these genes were not considered important for enhancing heterologous protein expression and, thus, Ferrari would lead away from the invention as well as failing to provide a reasonable expectation of success for it.

(c) Surprising and superior results obtained by deleting *rocR* or *sigL*. Lastly, assuming, *arguendo*, that the Examiner had established a *prima facie* case for obviousness, the Appellants have shown the surprisingly superior effects of deleting *rocR* or *sigL* on the expression of heterologous proteins compared to wild-type strains or other mutated strains containing these genes, see Table 4 on page 26 of the specification (reproduced below):

Table 4

Name of deleted gene	Gene ID	Gene size (bp)	Size of deleted fragment (bp)	Amount of produced (secreted) alkaline amylase (relative value)
Cultured for 3 days				
slr	BG11858	459	394	178
treR	BG11011	717	656	124
yopO	BG13648	213	169	364
yvaN	BG14069	408	379	148
yvbA	BG14078	273	210	171
None (Wild type)	—	—	—	100
Culture for 5 days (Wild type)				
cspB	BG10824	204	171	195
rocR	**BG10723**	**1386**	**1359**	**215**
sigL	**BG10748**	**1311**	**1256**	**204**
glcT	BG12593	858	811	132
yvdE	BG12414	951	916	127
yacP	BG10158	513	513	110
None (Wild type)	—	—	—	100
Cultured for 6days				
yycH	BG11462	1368	1368	120
licR	BG11346	1926	1889	122
None (Wild type)	—	—	—	100

Deletion of *sigL* or *rocR* more than doubled the yield of heterologous protein. This would not have been predictable from the prior art for the reasons above and in view of the

results shown in Ferarri's Figs. 7 and 8. ". . .evidence of criticality or unexpected results, commercial success, long-felt but unsolved needs, failure of others, skepticism of experts, etc., must be considered by the examiner in determining the issue of obviousness of claims for patentability under 35 U.S.C. 103."; MPEP 716.01.

Consequently, this rejection cannot be sustained because the Examiner has not established a prima facie due to lack of any suggestion or reasonable expectation of success for the invention in the prior art and in view of the surprisingly high enhancement of heterologous protein yield achieved by selectively deleting *rocR* or *sigL*.

RELIEF REQUESTED

The Appellants respectfully request reversal of the grounds of rejection above and the allowance of this application.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.
Stephen G. Baxter

Thomas M. Cunningham
Registration No.: 45,394

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413 -2220
(OSMMN 07/09)